Prolonged-release fampridine in multiple sclerosis: Improved ambulation effected by changes in walking pattern

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Abstract: BACKGROUND Prolonged-release fampridine (PR-fampridine, 4-aminopyridine) increases walking speed in the timed 25-foot walk test (T25FW) in some patients (timed-walk responders) with multiple sclerosis (MS). OBJECTIVE To explore the effects of PR-fampridine on different aspects of walking function and to identify associated gait modifications in subjects with MS. METHODS In this prospective, randomized, placebo-controlled, double-blind, phase II study (FAMPKIN; clinicaltrials.gov, NCT01576354), subjects received a 6-week course of oral placebo or PR-fampridine treatment (10 mg, twice daily) before crossing over. Using 3D-motion-analysis, kinematic and kinetic parameters were assessed during treadmill walking (primary endpoint). Clinical outcome measures included T25FW, 6-minute walk test (6MWT), and balance scales. Physical activity in everyday life was measured with an accelerometer device. RESULTS Data from 55 patients were suitable for analysis. Seventeen subjects were timed-walk responders under PR-fampridine. For the total study population and for responders, a significant increase in walking speed (T25FW) and distance (6MWT) was observed. Gait pattern changes were found at the single-subject level and correlated with improvements in the T25FW and 6MWT. Physical activity was increased in responders. CONCLUSION PR-fampridine improves walking speed, endurance, and everyday physical activity in a subset of subjects with MS and leads to individual modifications of the gait pattern.

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Abstract

Background: Prolonged-release fampridine (PR-fampridine, 4-aminopyridine) increases walking speed in the timed 25-foot walk test (T25FW) in some patients (timed-walk responders) with multiple sclerosis (MS).

Objective: To explore the effects of PR-fampridine on different aspects of walking function and to identify associated gait modifications in subjects with MS.

Methods: In this prospective, randomized, placebo-controlled, double-blind, phase II study (FAMPKIN; clinicaltrials.gov, NCT01576354) subjects received a 6-week course of oral placebo or PR-fampridine treatment (10 mg, twice daily) before crossing over. Using 3D-motion-analysis, kinematic and kinetic parameters were assessed during treadmill walking (primary endpoint). Clinical outcome measures included T25FW, 6-minute walk test (6MWT) and balance scales. Physical activity in everyday life was measured with an accelerometer device.

Results: Data from 55 patients were suitable for analysis. Seventeen subjects were timed-walk responders under PR-fampridine. For the total study population and for responders, a significant increase in walking speed (T25FW) and distance (6MWT) was observed. Gait pattern changes were found at the single-subject level and correlated with improvements in the T25FW and 6MWT. Physical activity was increased in responders.

Conclusion: PR-fampridine improves walking speed, endurance and everyday physical activity in a subset of subjects with MS and leads to individual modifications of the gait pattern.

Introduction

In multiple sclerosis (MS), walking impairment is a common consequence of the immune-mediated inflammatory destruction of myelin sheaths, axons and neurons in the human central nervous system (CNS). Ambulatory function is rated as most valuable by patients with early and advanced MS while subclinical gait abnormalities can be found even in the early stages of the disease[1, 2].

MS lesions can occur at different locations within the CNS and may affect the anatomical systems important for walking and motor control to varying degrees[3]. Resultant symptoms, including paresis, sensory deficits, spasticity and fatigue, interfere with different aspects of walking function such as gait velocity, endurance and balance. Consequently, MS represents a particularly heterogeneous disease even at the level of a single, albeit multi-factorial, symptom such as walking dysfunction.

At present, various disease-modifying immunotherapies for MS are available, but treatment options to specifically improve MS-related walking disturbances remain limited[4, 5]. Two phase III trials showed that oral administration of prolonged-release fampridine (PR-fampridine; dalfampridine) increases walking speed in the timed 25-foot walk test (T25FW) in a subset of patients with MS (timed-walk responders)[6-8]. The active substance 4-aminopyridine (4-AP) leads to improved signal conduction in demyelinated axons via the blockade of voltage-gated potassium channels[9]. This increase in walking speed was first observed two weeks after the initiation of treatment, was clinically relevant as indicated by a patient-based walking scale (12-item multiple sclerosis walking scale, 12-item WS) and was independent of demographic factors or the type of MS[8, 10, 11]. However, it remains unclear how this increased walking speed was achieved, e.g. through improved strength or balance, or reduced motor fatigue.

The objective of the present investigator-initiated phase II trial ("FAMPKIN") was (1) to further characterize the effects of PR-fampridine on different aspects of walking function in

subjects with MS, (2) to analyze the relevance of PR-fampridine-induced improvements in the patients` daily life and (3) to identify the modification of gait patterns underlying the beneficial effects of PR-fampridine.

Patients and Methods

Study participants. Sixty-one outpatients (**Figure 1 A**) aged 18 to 65 years with a diagnosis of relapsing-remitting (RRMS), primary- (PPMS) or secondary-progressive (SPMS) MS[12] were included (**Table 1**). Patients were recruited at the University Hospital Zurich, Switzerland in 2012 and 2013. Participants had a clinically apparent walking impairment (e.g., ataxia, paresis of leg muscles or restricted walking duration) but were able to cover a distance of at least 50 meters within 6 minutes with or without walking aids. Patients with a history of seizure, prior exposure to 4-AP, or other conditions impeding gait, such as cardiac, pulmonary or orthopaedic diseases, were excluded. Subjects who experienced MS relapses or whose MS therapy changed during the study were excluded from final analyses. Changes in concomitant medication and adverse events (AEs) were recorded at each visit. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the Good Clinical Practice guidelines and approved by the Zurich cantonal ethics committee and Swissmedic. All participants gave written informed consent. Subjects did not receive payment for participation in the trial. The trial, named FAMPKIN, was registered at clinicaltrials.gov (NCT01576354). Monitoring was conducted by the Clinical Trials Center of the University Hospital Zurich, Switzerland.

Study design. This explorative study was a prospective, single-center, randomized, placebo-controlled, double-blind, phase II trial with a crossover design (**Figure 1 B**). After screening, 13 study visits took place including five baseline visits (visits 0, 1, 2, 7 and 12). After a single-blind, placebo run-in period of two weeks, subjects were randomized at a 1:1 ratio to receive double-blind treatment with either placebo or 10 mg PR-fampridine every 12 hours for six weeks. Patients then all received single-blind placebo treatment for two weeks (wash-out period), followed by another six weeks of double-blinded treatment. Subjects who received PR-fampridine during the first double-blind treatment period received placebo in the

second period and vice versa (crossover). A two-week observation period was maintained after the second treatment period. Blinding and randomization was performed by the Zurich cantonal pharmacy. All subjects and staff were blinded to treatment assignments. PR-fampridine matrix tablets and matching placebo tablets were provided by Biogen. Subject treatment compliance was confirmed by counting the tablets remaining in the returned boxes.

Outcome measures. Clinical assessments, questionnaires, activity monitoring and gait analyses were performed at different time points as illustrated in **Supplementary Table 1**. Clinical walking and balance tests including the T25FW, 6-minute walk test (6MWT), the timed up-and-go test (TUG), Berg balance scale (BBS) and dynamic gait index (DGI) and the lower extremity manual muscle test (LEMMT) were conducted as reported previously[6, 7, 13-17]. Subjects who used walking aids in the clinical tests at screening continued to use the same devices for all subsequent tests. Timed-walk responders were subjects with a faster walking speed in the T25FW for at least three of the four visits during the double-blind treatment periods as compared with the maximum speed achieved in the five baseline visits[6, 7]. Patients' perception of their treatment's effects was evaluated using the 12-item WS for walking function (maximal score of 60 points)[18] and the Wurzburg Fatigue Inventory for Multiple Sclerosis for motor and cognitive fatigue (WEIMuS; maximal score for cognitive fatigue 36 points, motor fatigue 32 points)[19].

Subjects were asked to wear an accelerometer device (Actiwatch 2, Philips Respironics, USA) attached to the ankle of the more impaired leg (based on the neurological examination) for 14 consecutive days during each of the two double-blind treatment phases and to keep a sleep diary over this period. The device was a small piezo-electric acceleration sensor with a sensitivity of 0.025 G and a sampling rate of 32 Hz. Using the Actiware software (V6.0.1), motion was measured in activity counts averaged over 15-second intervals[20].

Three-dimensional gait analysis was conducted while walking on an instrumented treadmill (120 Hz, FDM-THM-M System, zebris Medical GmbH, Germany) using 14 infrared cameras

(Vicon, UK) with a sampling rate of 200 Hz. Small reflective markers attached to the subjects' skin overlying anatomical landmarks at the trunk, the upper and lower extremities allowed for 3-dimensional reconstruction of movements using a full-body gait model (Plug-in-Gait, Vicon, UK). Vertical ground reaction force data were exported from the treadmill's integrated capacitive pressure platform[21] normalized to body weight and the area under the force curve determined using a custom-made Matlab script (Mathworks, Inc., USA). Gait analysis was performed twice during each double-blind treatment period (**Supplementary Table 1**). Subjects walked barefoot on the horizontal treadmill and were continuously secured by a fall-stop system. Treadmill speed was set to 80% of the mean maximal overground walking speed as assessed in the T25FW at Visit 0 and 1. Treadmill speed and other conditions (e.g., use of handrails) were kept constant for all sessions for each subject. Gait was assessed over a period of 30 seconds per visit (10-20 step cycles). Analysis comprised 15 of the most relevant kinematic and kinetic gait parameters and was conducted in Vicon Nexus (V1.8.5) and ProCalc (Vicon, UK)[2, 22-24]. A significant change in kinematic parameters under treatment with PR-fampridine was defined as the study's primary endpoint. T25FW (including responder status), 6MWT, TUG, BBS, DGI, LEMMT, 12-item WS, WEIMuS, accelerometer data and sleep duration were defined as secondary outcomes.

Safety assessments included a physical examination, resting electrocardiogram, vital signs and laboratory tests comprising haematology, blood chemistry and urine analysis (**Supplementary Table 1**).

Statistics. Statistical analysis was performed with SPSS (V21, IBM Corp., USA), GraphPad Prism 5 (V5.01, GraphPad Software, Inc., USA) and Matlab (Mathworks, Inc., USA). For data presentation, groups of *all subjects*, *fampridine-responders* and *fampridine- and placebo-non-responders* were designated. Fisher's exact test was applied to determine significant differences in the incidence of AEs under treatment with placebo and PR-fampridine. For clinical gait measures, activity parameters, fatigue, sleep duration, kinematic and kinetic

parameters, two-tailed, paired *t*-tests were performed to detect differences between placebo and PR-fampridine treatment. Holm's sequential Bonferroni procedure was applied to adjust for multiple comparisons. Repeated measures one-way ANOVA was used to analyse changes of walking speed (T25FW) over time in the responder subgroup. To reduce dimensionality of the dataset, linearly uncorrelated components (principal components, PCs) were identified using an alternating least-squares algorithm on the centred and standardized dataset with Kaiser-Guttman stopping criteria[25]. Each data-point subsequently underwent orthogonal linear transformation to determine its loading in PC-space. Translation between treatment conditions in the PC-space was quantified for each data pair (patient treated with placebo or PR-fampridine) in each dimension and group clustering was characterized through volume shifts in a fitted convex hull. To test for differences of translations between treatments, a two-tailed paired *t*-test was applied. Changes of kinematic parameters at the single-subject level were evaluated by calculating the between-measurement variability (measurement error) within each double-blind treatment period for each parameter. Between-measurement variability under treatment with PR-fampridine and placebo was similar (**Supplementary Table 2**), therefore an overall mean between-measurement variability was determined for each parameter. Changes of kinematic parameters under treatment with PR-fampridine compared to placebo exceeding the threshold of the between-measurement variability ± 2 standard deviations were defined as relevant.

Results

We assessed 131 subjects with MS for eligibility, of whom 64 subjects were screened and 61 randomly assigned to treatment (**Figure 1 A**). One subject withdrew before completing the second double-blind treatment period. Sixty patients completed the trial. Five subjects were excluded from final analysis (one MS relapse, one subject broke his leg and, three noncompliance with the study drug). Data from 55 patients was thus available for analysis. Oral administration of PR-fampridine for six weeks was well tolerated and showed no significant side-effects compared to placebo treatment (**Table 2**). In the T25FW, 17 of 55 subjects (31%) met the timed-walk responder criterion[6, 7] under treatment with PR-fampridine (fampridine-responders; **Figure 2**). The number of responders was similar in both study arms (8 vs. 9 subjects). Three subjects met the responder criterion only under placebo (placebo-responders) while five did so during both treatment periods (double-responders); these groups were not classified as responders. Thirty subjects were classified as non-responders (55%). Compared to baseline performance, responders showed a mean increase of walking speed of 14% in the T25FW under PR-fampridine. This gain was significantly higher than the 2% increase in walking speed observed under placebo (p<0.0001) and was already present one week after the initiation of treatment (**Figure 2 B, C**). Significantly increased walking speed under PR-fampridine persisted as an effect when determined for the entire study population (p<0.0001; 9% change from baseline), but was not evident in the non-responder group when analyzed alone. Walking distance in the 6MWT increased significantly for all patients under treatment with PR-fampridine (p<0.0001 compared to placebo; 4% change from baseline; **Figure 2 D**). This effect was more pronounced in the responders (p=0.0004 compared to placebo; 8% change from baseline). Treatment with PR-fampridine did not induce significant changes in the other clinical tests comprising the TUG (**Figure 2 E**), LEMMT, BBS and DGI or in the 12-item WS (**Supplementary Figure 1**). For the total study population, no significant changes in cognitive or motor fatigue were detected during treatment with PR-

fampridine (**Supplementary Figure 2 A, B**). There was a trend towards a reduction in cognitive (p=0.253) and, especially, motor (p=0.0534) fatigue in the responders under treatment with PR-fampridine. Sleep duration tended to be slightly shorter (about 12 minutes per day in the total study population; p=0.0294) under PR-fampridine (**Supplementary Figure 2 C**).

One subject declined to wear the accelerometer, leaving 54 datasets for analysis. Physical activity assessed with this device was not significantly altered by PR-fampridine in the total study population, but showed a significant increase in activity counts per day during the waking phase in responders (p=0.0045; **Figure 3 A, B**). The cumulative time spent active per day did not differ in subjects between PR-fampridine and placebo treatment in any group, indicating that the increase in activity counts is driven by intensity rather than duration of physical activity in responders (**Figure 3 C**).

One subject was unable to walk on the treadmill at 80% of his overground T25FWT speed and was excluded from the kinematic and kinetic analysis (n=54). Compared to placebo, averaged group values of most of the kinematic and kinetic measures assessed during treadmill walking were not altered by PR-fampridine (**Table 3**). In the total study population, there was a statistically significant increase of knee range of motion (ROM) in the more (MIL; p<0.0001) and the less impaired (LIL; p=0.0006) leg during treatment with PR-fampridine. However, these changes were small, with a difference in ROM of only 2 degrees. Alternating least-squares identification of principal components (PC) revealed three components with eigenvalues greater than 1 which together accounted for about 90% of the total variance (**Supplementary Figure 3**). Translation between treatments was not significantly different between groups on any component. Point dispersion and centroid location was similar for placebo and PR-fampridine treatment. Thus, PC analysis showed no significant changes under treatment with PR-fampridine in comparison to placebo at the group-level.

At the single-subject level, we observed heterogeneous fampridine-induced modifications of the gait pattern in a subset of patients. Some individuals showed a strongly increased ROM in the knee under PR-fampridine (**Figure 4 A-C**; subject #31 in **Figure 5 A, B**) while other subjects, for instance, demonstrated a greater ROM in the hip joint (subject #9 in **Figure 5 A, B**). Using the between-measurements variability ± 2 standard deviations as a threshold, single-subject analysis showed that 25 of the 54 subjects (46%) demonstrated relevant changes in at least one gait parameter (**Figure 5 B**). Type and magnitude of modifications differed considerably across subjects and were not restricted to the more or the less impaired leg. Proportionally more subjects with at least one altered parameter under PR-fampridine were in the responder than the non-responder subgroup (**Figure 5 C**). Subjects with at least one changed kinematic parameter under treatment with PR-fampridine demonstrated significantly larger increases of walking speed in the T25FW (two-tailed unpaired *t*-test: p=0.0066, **Figure 5 D**) and walking distance in the 6MWT (two-tailed unpaired *t*-test: p=0.0114, **Figure 5 E**) than subjects without such gait pattern changes, indicating that walking improvements under PR-fampridine are based on individual modifications of the gait pattern.

Discussion

The objective of this explorative, investigator-initiated phase II trial was to characterize the effects of PR-fampridine on different aspects of walking impairment in subjects with MS, using a comprehensive gait analysis protocol including clinical tests, questionnaires and biomechanical parameters. Since MS is a chronic condition and PR-fampridine has a half-life in the range of a few hours[26], we chose a crossover study design to compensate for the limited number of eligible subjects in this single-center trial. A wash-out period of two weeks appeared to be sufficient to avoid carryover effects (see **Figure 2 B**).

We found that roughly one third of the patients met the timed-walk responder criterion defined by Goodman *et al*. under treatment with PR-fampridine, in line with the results from the previous pivotal trials[6, 7]. Walking speed increased in responders by an average of 14% in the T25FW under treatment with PR-fampridine, a somewhat smaller effect than the 20-25% increase reported for responders in the larger pivotal trials[8]. This difference in effect sizes may explain the absence of a significant change in the related patient-based outcome measure of walking function. In the previous trials, the 12-item WS was used to show that the effects of PR-fampridine were clinically meaningful[6, 7, 10, 11]. In the present study, clinical relevance of the observed improvements was demonstrated by the accelerometer data, showing for the first time that treatment with PR-fampridine enhances physical activity in everyday life in responders. However, the type of physical activity (e.g. walking, running, climbing stairs) that increased with PR-fampridine could not be determined with our device. We also demonstrated that PR-fampridine not only increased maximal walking speed, but also the walking distance achieved in the 6MWT, indicating improved walking endurance[27]. There was no effect of the study drug on static and dynamic balance, which is in contrast to findings from the recent MOBILE trial[28]. Factors limiting the use of the BBS and the DGI scores as outcome measures in the present study may include its small sample size (n=55) and the low sensitivity and inherent ceiling effects of such scales.

The main objective of the present study was to uncover the functional mechanisms that drive improvements in the T25FW and 6MWT. We used gold standard 3D-gait analysis to identify mechanisms underlying gait disturbances[29]. There was only a single common effect of PR-fampridine treatment; a significant increase of the ROM in the knee joint. However, this ROM change was minor, casting doubt on its clinical relevance and contribution to the observed improvements. Using a descriptive statistical approach based on the between-measurement variability to evaluate treatment effects at the single-subject level, we observed extensive but heterogeneous alterations of the biomechanical parameters in about half of subjects. The incidence of gait parameter changes significantly correlated with improvements in the T25FW and 6MWT under treatment with PR-fampridine. Subjects with MS can experience a variety of neurological impairments and this heterogeneity may also be reflected in MS-related gait disturbances where different aspects of ambulatory function can be affected to varying degrees. In our cohort, subjects were affected with a wide range of gait disturbances characterized by, for example, spasticity, sensory and cerebellar ataxia, or weakness of the leg muscles. Therefore, the individual kinematic adaptations observed in our data during treatment with PR-fampridine may be anchored in both deficit-specific improvements and individual compensatory strategies.

Finally, the safety data of our trial confirmed good tolerability of PR-fampridine[6, 7]. In the earlier trials, urinary tract infections (UTIs) occurred more frequently in patients treated with PR-fampridine in comparison to placebo[6, 7]. We did not observe a higher incidence of UTIs, in line with the findings of a recently published *post hoc* analysis[30].

To conclude, our findings indicate that treatment with PR-fampridine is well tolerated and improves walking speed, endurance and physical activity in a subset of subjects with MS. Improvements of ambulatory function are achieved through individual modification of the gait pattern.

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Conflict of Interest Statement

BZ, AC, MW and ML received honoraria, travel grants and funding from Biogen. AC and ML are consultants to Biogen. Since June 2015, MF is an employee of Biogen. The other authors declare no competing financial interests.

Author Contributions

BZ and KR planed and designed the study and analyzed data. BZ and LF produced the figures and prepared the manuscript. LF, SK, LL, TS, DW and MF collected, analyzed and interpreted the data. BZ and CSE analyzed the data and performed statistics. AC participated in the enrolment and assessment of study participants. MW and ML conceived and supervised the study and critically revised the manuscript.

Responsibility

The FAMPKIN study was an investigator-initiated trial. The authors bear full responsibility for the study design, study conduction, data analysis and interpretation and the manuscript. BZ and ML had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Figure and Table legends

Figure 1. Patient disposition and study design.

(**A**) Flow diagram indicating number of patients assessed, screened, enrolled, randomized and analyzed. Reasons for early termination and exclusion from final analysis are given in the white boxes. (**B**) Study design and time schedule. Study visits are indicated by circles. Abbreviations: S, Screening Visit.

Table 1: Patient demographics and disease characteristics.

Abbreviations: SD, standard deviation; EDSS, expanded disability status scale.

Table 2: Summary of adverse events (AE) and serious adverse events (SAE).

Absolute numbers of AEs that occurred during 6 weeks of double-blind placebo and PR-fampridine treatment in 58 subjects are presented. Incompliant subjects were excluded from this analysis The total number of AEs for the entire study was 224. The most frequent AEs (without SAEs) occurring more than once in fampridine-treated patients are listed using MedDRA (Medical Dictionary for Regulatory Activities) terms. Statistical analysis demonstrated no difference between placebo and PR-fampridine treatment. Abbreviations: AE, adverse event; SAE, serious adverse event; NSTEMI, non-ST-segment-elevation myocardial infarction.

Figure 2. Clinical gait tests.

(**A**) Number and percentage of patients who classified as responder based on the T25FW test results during the two double-blind treatment periods. (**B**) Results of the T25FW test in the fampridine-responder subgroup (n=17) illustrated for each double-blind treatment visit and the two study arms (grey: PR-fampridine treatment until Visit 6, followed by placebo until Visit 11, n=8; black: placebo until Visit 6, followed by PR-fampridine until Visit 11, n=9). The single-blind placebo washout phase between Visit 6 and Visit 7 is not shown. Circles

represent group mean values for each time point ± standard error of the mean. For differences between time-points, repeated measures one-way ANOVA was performed for each arm separately. (**C-E**) Results of the T25FW, 6MWT and TUG for all patients (n=55), responders (n=17) and non-responders (n=30). Bars show group mean values ± standard error of the mean. Asterisk indicates significant differences. Abbreviations: Non-Resp., non-responder; T25FW, timed 25-foot walk test; 6MWT, 6-minute walk test; TUG, timed up-and-go test.

Figure 3. Activity during everyday life as assessed with an accelerometer device.

(**A**) Day-night-activity profile of a responder (subject #48, 42-years-old female with two young children, diagnosis of relapsing remitting MS, EDSS 3.5) over 11 consecutive days during the two double-blind treatment periods (left, placebo; right, PR-fampridine). Sleep phases are indicated by a dark grey background. Black background marks periods during which the device was not worn. Activity counts are illustrated as horizontal black lines. Scale bar, 1000 activity counts. (**B**) Cumulative daily activity counts for all subjects (n=54), responders (n=17), and non-responders (n=29). (**C**) Percentage of time with activity during the waking phase. Patient groups as in B. (**B**, **C**) Bars show group mean values for placebo (white) and PR-fampridine treatment (black) ± standard error of the mean. Asterisk indicates significant differences. Abbreviations: Mo, Monday; Tu, Tuesday; We, Wednesday; Th, Thursday; Fr, Friday; Sa, Saturday; Su, Sunday; Non-Resp., non-responder; s, seconds.

Table 3. Kinematic and kinetic parameters assessed during treadmill walking.

Results for different gait parameters are shown for all subjects, responders, and non-responders (non-resp.) and expressed as group mean values ± standard deviation. Significant differences between treatment with placebo and PR-fampridine are highlighted (bold and underlined). Abbreviations: MIL, more impaired leg; LIL, less impaired leg; AUC, area under the curve; ns, not significant; sig, significant.

Figure 4. Kinematics of a responder during treadmill walking at 80% of maximal overground walking speed.

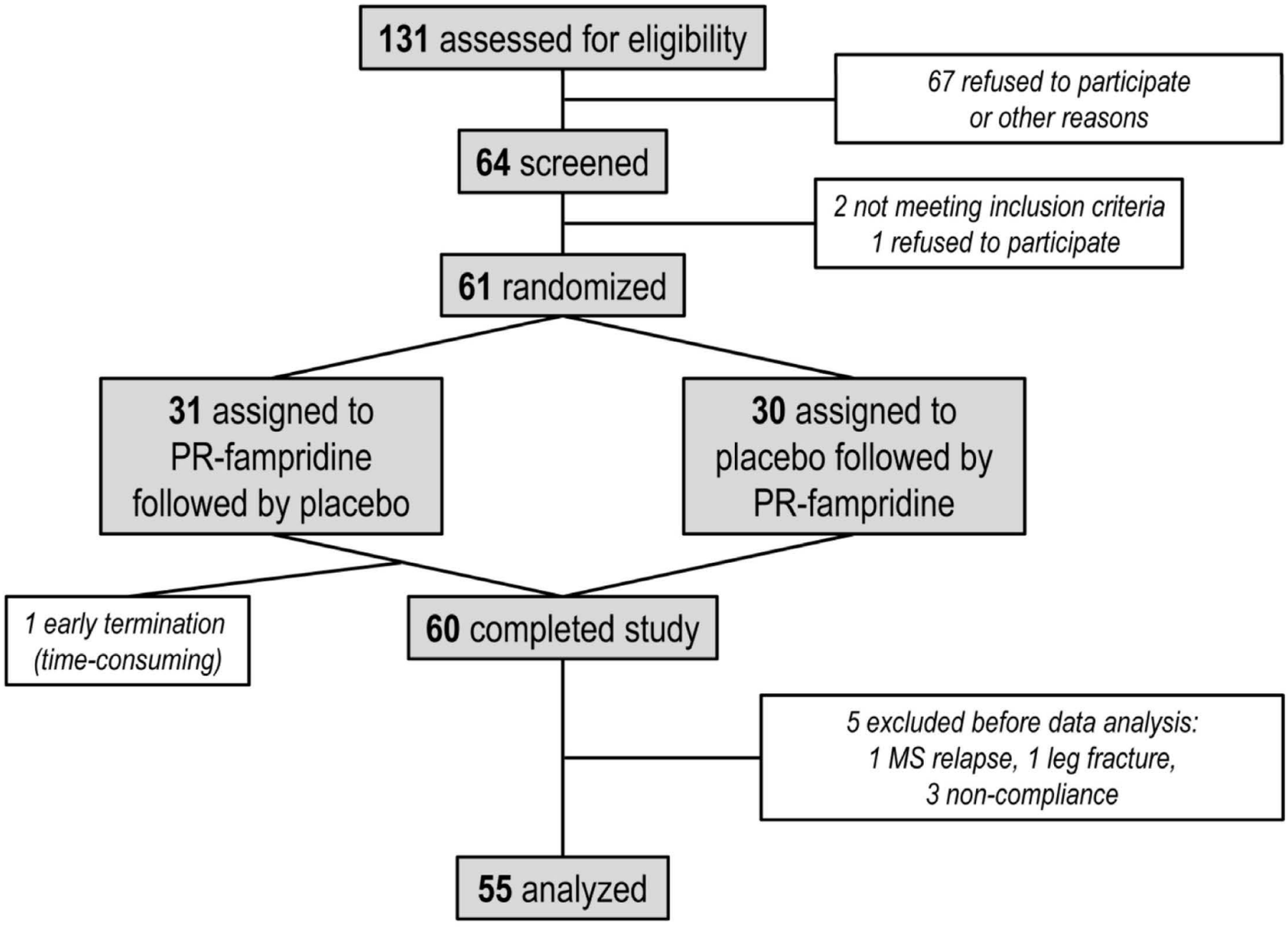
(**A**) Averaged sagittal angle trajectory of hip, knee and ankle joints of the more impaired leg assessed twice during each of the double-blind treatment periods with placebo (P1, P2; blue) and PR-fampridine treatment (F1, F2; red). (**B**) Averaged toe trajectory during treadmill walking under placebo and PR-fampridine as in (A). (**C**) Stick diagram of the more impaired leg illustrates limb excursions during the stance and swing phase under treatment with placebo (upper) and PR-fampridine (lower). Continuous arrows indicate direction of the treadmill; dotted arrows indicate the direction of leg movement. Abbreviations: deg, degrees.

Figure 5. Kinematic parameters assessed during treadmill walking for single subjects.

(**A**) Colour-coded changes in percent of different kinematic gait parameters (columns) under PR-fampridine in comparison to placebo are shown for each subject (rows). Responders are indicated by red patient numbers on the right. (**B**) Illustration of gait changes under treatment with PR-fampridine compared to placebo for each subject and parameter based on the variability between measurements within the same treatment phase. Increases (red) or reductions (blue) that are higher or lower than the calculated and averaged variability between measurements (measurement error) ± 2 standard deviations are shown. (**C-E**) Correlation between changes of the gait pattern (as defined in B), responder status and clinical gait tests. Asterisk indicates significant differences. Abbreviations: MIL, more impaired leg; LIL, less impaired leg; ROM, range of motion; DL, double-limb; BOS, base of support; COV, coefficient of variation; SD, standard deviation; Non-Resp., non-responder.

Figure 1

A


131 assessed for eligibility
67 refused to participate or other reasons
64 screened
2 not meeting inclusion criteria
1 refused to participate
61 randomized
31 assigned to PR-fampridine followed by placebo
30 assigned to placebo followed by PR-fampridine
1 early termination (time-consuming)
60 completed study
5 excluded before data analysis:
1 MS relapse, 1 leg fracture,
3 non-compliance
55 analyzed

B

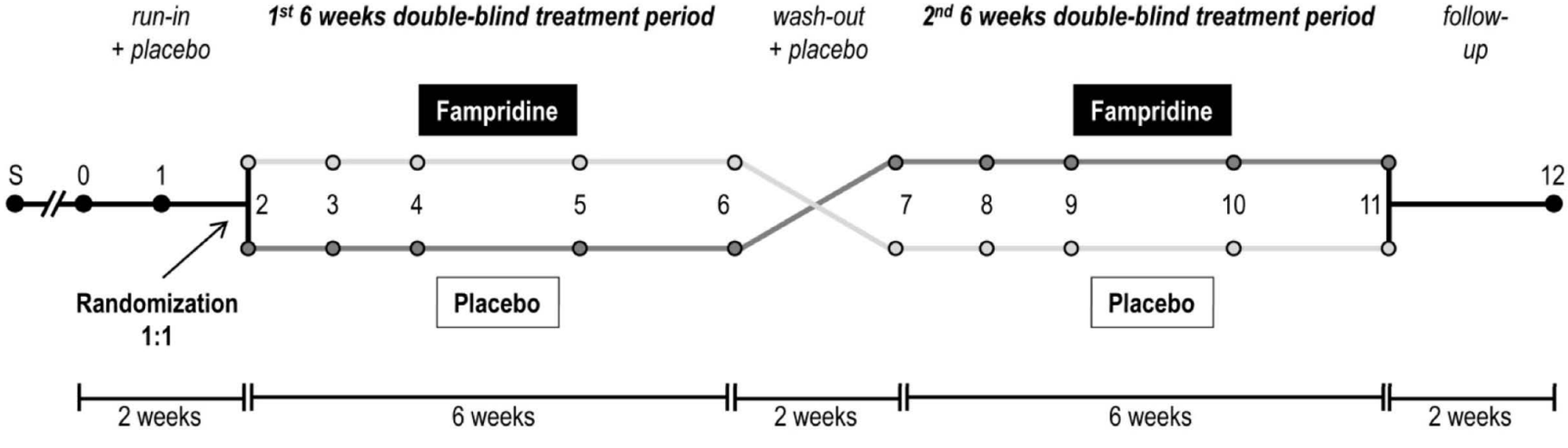

run-in + placebo
1st 6 weeks double-blind treatment period
wash-out + placebo
2nd 6 weeks double-blind treatment period
follow-up
Fampridine
Fampridine
S
0
1
2
3
4
5
6
7
8
9
10
11
12
Randomization 1:1
Placebo
Placebo
2 weeks
6 weeks
2 weeks
6 weeks
2 weeks

Figure 2

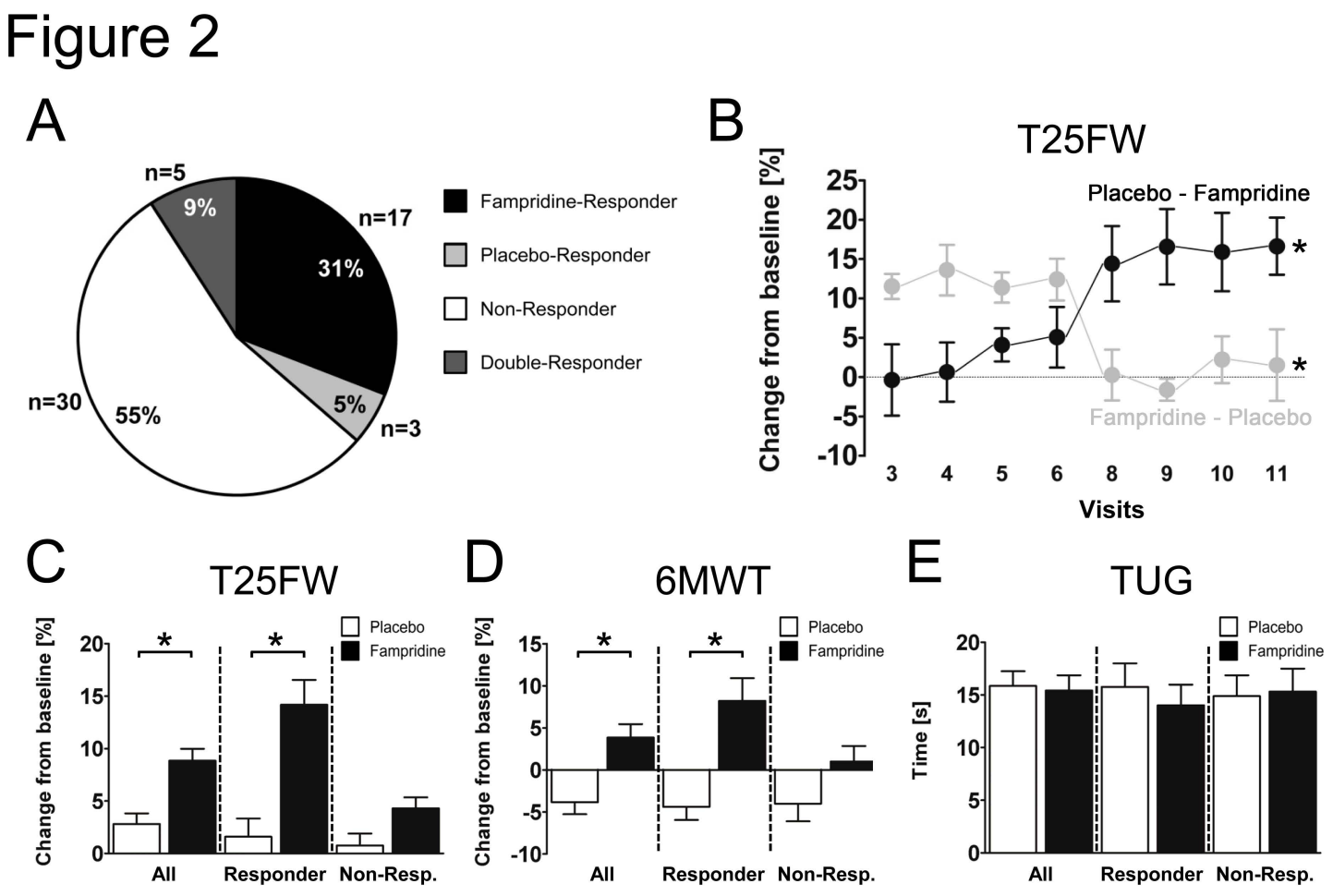

A
n=5
9%
n=17
31%
n=30
55%
5%
n=3
Fampridine-Responder
Placebo-Responder
Non-Responder
Double-Responder
B
T25FW
Placebo - Fampridine
Fampridine - Placebo
Change from baseline [%]
25
20
15
10
5
0
-5
-10
3
4
5
6
8
9
10
11
Visits
*
*
C
T25FW
Change from baseline [%]
Placebo
Fampridine
All
Responder
Non-Resp.
D
6MWT
E
TUG
Time [s]

Figure 3

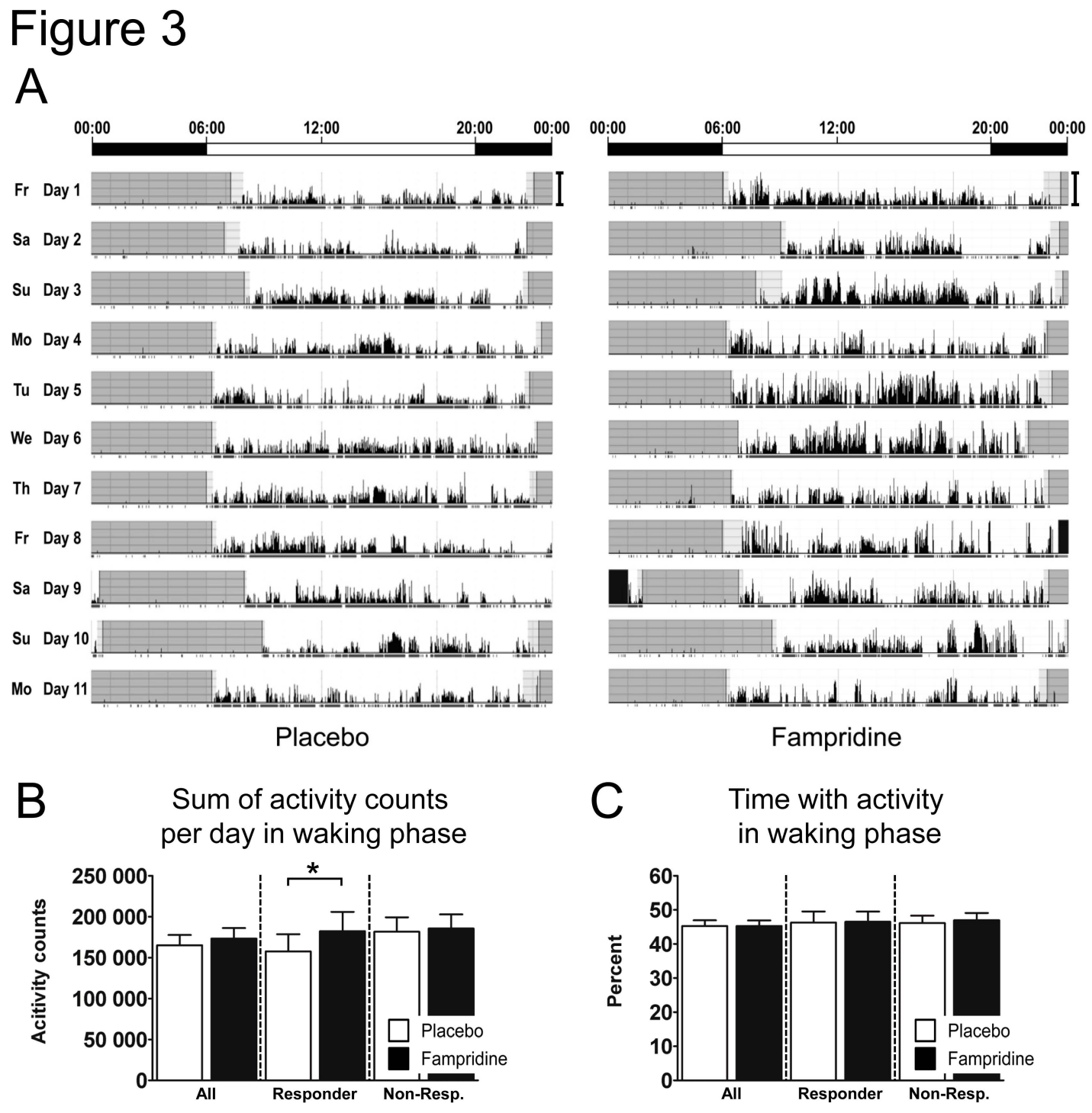

A
00:00
06:00
12:00
20:00
00:00
Fr Day 1
Sa Day 2
Su Day 3
Mo Day 4
Tu Day 5
We Day 6
Th Day 7
Fr Day 8
Sa Day 9
Su Day 10
Mo Day 11
Placebo
Fampridine
B
Sum of activity counts per day in waking phase
Acitivity counts
250 000
200 000
150 000
100 000
50 000
0
*
All
Responder
Non-Resp.
Placebo
Fampridine
C
Time with activity in waking phase
Percent
60
50
40
30
20
10
0
All
Responder
Non-Resp.
Placebo
Fampridine

Figure 4

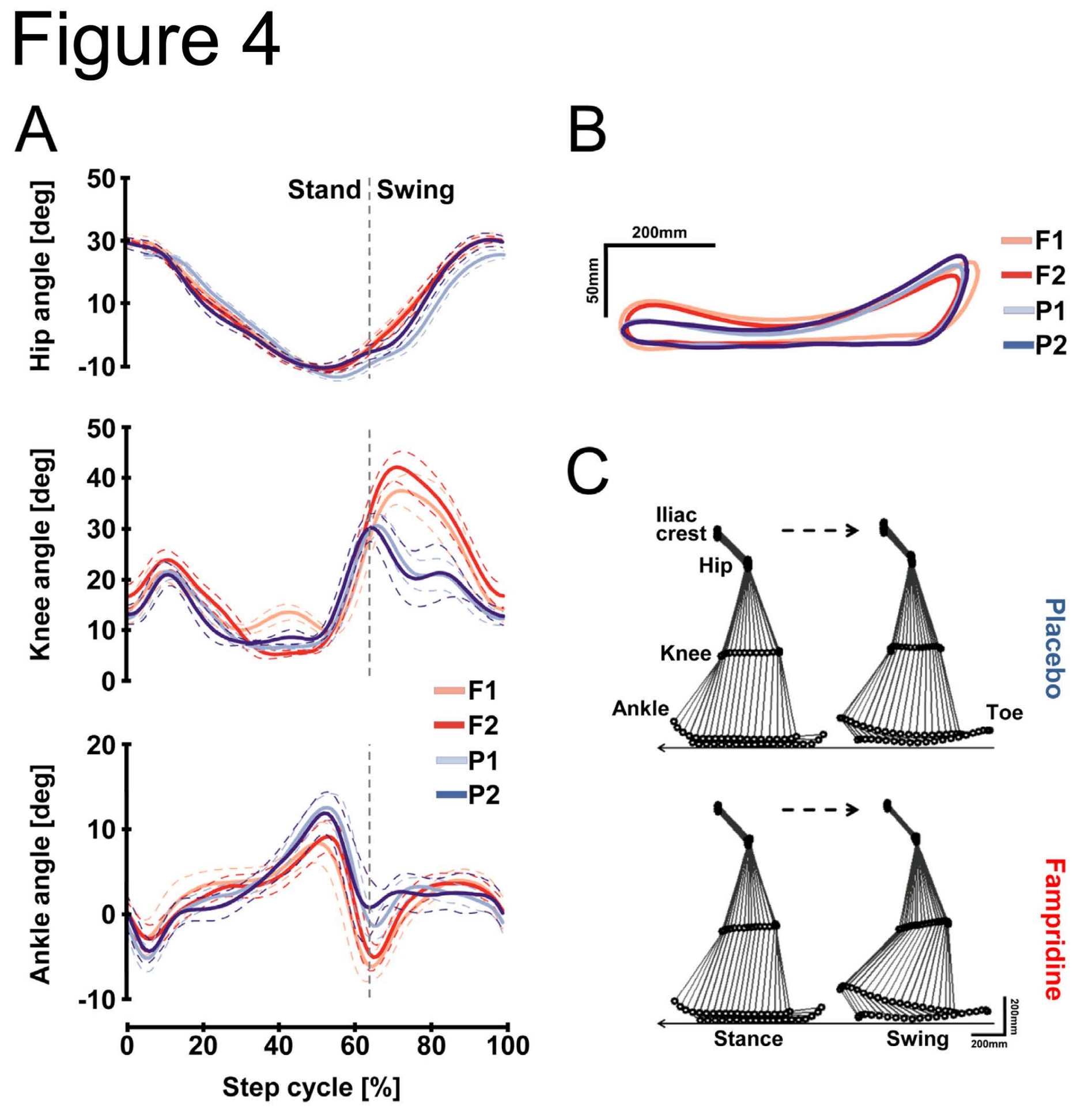
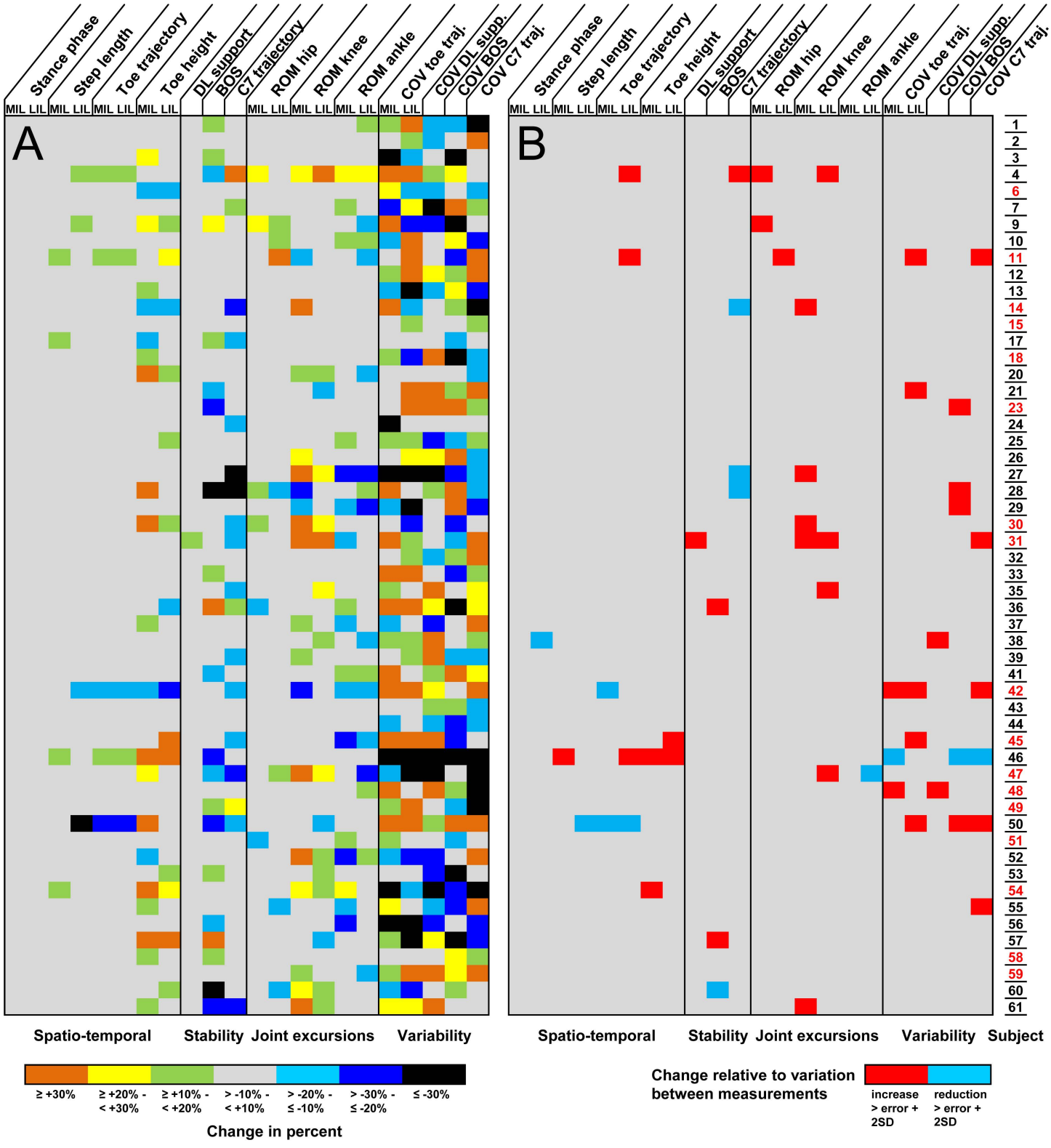
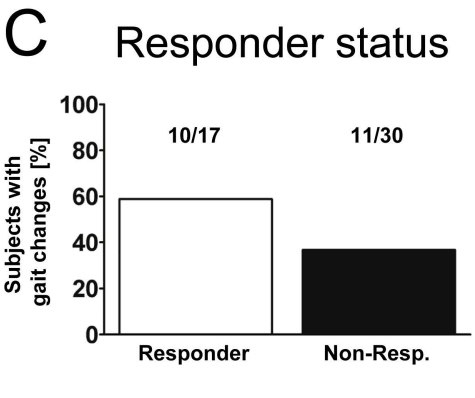
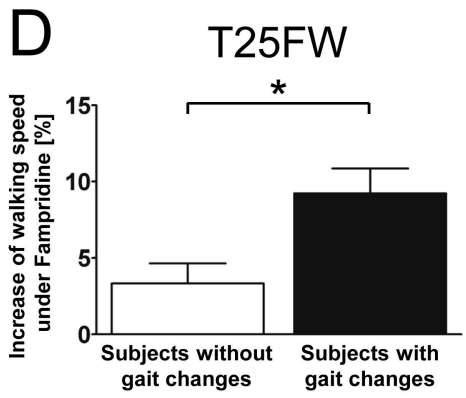

A
B
C
Stand
Swing
Hip angle [deg]
Knee angle [deg]
Ankle angle [deg]
Step cycle [%]
F1
F2
P1
P2
200mm
50mm
Iliac crest
Hip
Knee
Ankle
Toe
Placebo
Fampridine
Stance
Swing

Figure 5


Stance phase
Step length
Toe trajectory
Toe height
DL support
BOS
C7 trajectory
ROM hip
ROM knee
ROM ankle
COV toe traj.
COV DL supp.
COV BOS
COV C7 traj.
MIL LIL
A
B
1 2 3 4 6 7 9 10 11 12 13 14 15 17 18 20 21 23 24 25 26 27 28 29 30 31 32 33 35 36 37 38 39 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61
Spatio-temporal
Stability
Joint excursions
Variability
Subject
≥ +30%
≥ +20% - < +30%
≥ +10% - < +20%
> -10% - < +10%
> -20% - ≤ -10%
> -30% - ≤ -20%
≤ -30%
Change in percent
Change relative to variation between measurements
increase > error + 2SD
reduction > error + 2SD


C
Responder status
Subjects with gait changes [%]
100
80
60
40
20
0
10/17
11/30
Responder
Non-Resp.


D
T25FW
Increase of walking speed under Fampridine [%]
15
10
5
0
*
Subjects without gait changes
Subjects with gait changes

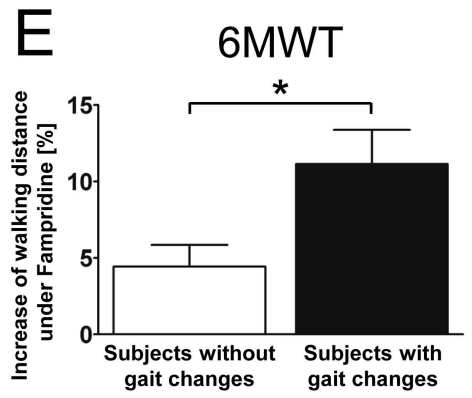

E
6MWT
Increase of walking distance under Fampridine [%]
15
10
5
0
*
Subjects without gait changes
Subjects with gait changes

Table 1: Patient demographics and disease characteristics.

Age in years, mean ± SD (range)		48.6 ± 9.8	(27 - 64)
Gender, number (%)	male	21	(38)
	female	34	(62)
Type of MS, number (%)	relapsing remitting (RRMS)	29	(53)
	primary progressive (PPMS)	5	(9)
	secondary progressive (SPMS)	21	(38)
Disease duration in years from diagnosis, mean ± SD (range)		11.9 ± 7.4	(1 - 37)
EDSS, mean ± SD (range)	at baseline	4.9 ± 1.3	(2.5 - 6.5)
	at last visit	5.1 ± 1.3	(2.5 - 6.5)
Concomitant MS treatment, number (%)	total	34	(62)
	with an interferon	9	(16)
	with glatiramer acetate	2	(4)
	with natalizumab	21	(38)
	with fingolimod	2	(4)

Table 2: Summary of adverse events (AE) and serious adverse events (SAE).

	Placebo (6 weeks)	Fampridine (6 weeks)
Total number of AEs	**67**	**74**
Severity		
Mild	45	54
Moderate	21	17
Severe	1	3
Relation to treatment		
Unlikely	8	6
Possible	59	68
Total number of SAEs	**1**	**2**
Myocardial infarction (subacute NSTEMI)	0	1
Atrial flutter	1	0
Ankle fracture	0	1
Most frequent AEs		
Nervous system disorders		
Total	**16**	**21**
Headache	5	7
Dizziness	4	3
Sciatica	2	3
Vertigo	1	2
Gastrointestinal disorders		
Total	**17**	**11**
Nausea	5	4
Abdominal pain	4	3
Renal and urinary disorders		
Total	**11**	**13**
Urinary tract infection	9	9
Respiratory, thoracic and mediastinal disorders		
Total	**7**	**8**
Cough	2	3
Nasopharyngitis	3	2
Investigations		
Total	**1**	**5**
Transaminases increased	1	2

Table 3. Kinematic and kinetic parameters assessed during treadmill walking.

Spatio-temporal gait parameters				
	Placebo	**Fampridine**	***P*-value**	**Sig.**
Duration of stance phase MIL (% of step cycle)				
All subjects (n=54)	67±5	67±5	0.0081	ns
Responder (n=17)	67±4	67±4	0.0920	ns
Non-Resp. (n=30)	66±5	66±5	0.0429	ns
Duration of stance phase LIL (% of step cycle)				
All subjects (n=54)	68±4	69±5	0.0824	ns
Responder (n=17)	68±4	69±4	0.1346	ns
Non-Resp. (n=30)	68±4	68±5	0.8192	ns
Step length MIL (mm)				
All subjects (n=54)	469±130	472±129	0.3305	ns
Responder (n=17)	474±112	478±115	0.4771	ns
Non-Resp. (n=30)	481±145	480±144	0.8070	ns
Step length LIL (mm)				
All subjects (n=54)	467±122	469±126	0.5731	ns
Responder (n=17)	484±96	481±100	0.5046	ns
Non-Resp. (n=30)	469±141	469±148	0.9185	ns
Length of toe trajectory MIL (mm)				
All subjects (n=54)	1258±238	1266±246	0.2912	ns
Responder (n=17)	1288±217	1292±222	0.8146	ns
Non-Resp. (n=30)	1268±266	1269±282	0.9084	ns
Length of toe trajectory LIL (mm)				
All subjects (n=54)	1288±252	1292±258	0.5866	ns
Responder (n=17)	1330±218	1330±230	0.9958	ns
Non-Resp. (n=30)	1297±278	1292±292	0.5128	ns
Toe height MIL (mm)				
All subjects (n=54)	52±25	54±24	0.0500	ns
Responder (n=17)	51±22	51±20	0.9671	ns
Non-Resp. (n=30)	57±27	59±27	0.0798	ns
Toe height LIL (mm)				
All subjects (n=54)	55±21	57±22	0.0223	ns
Responder (n=17)	55±19	57±21	0.2347	ns
Non-Resp. (n=30)	59±23	60±24	0.4162	ns

Measures of variability				
	Placebo	**Fampridine**	***P*-value**	**Sig.**
Coefficient of variation of length of toe trajectory MIL				
All subjects (n=54)	3.8±1.6	3.8±1.5	0.6703	ns
Responder (n=17)	3.3±1.2	3.8±1.2	0.0579	ns
Non-Resp. (n=30)	3.9±1.5	3.9±1.7	0.9235	ns
Coefficient of variation of length of toe trajectory LIL				
All subjects (n=54)	3.6±1.6	3.7±1.8	0.5756	ns
Responder (n=17)	3.3±1.4	3.6±1.4	0.5129	ns
Non-Resp. (n=30)	3.8±1.7	3.9±2.1	0.7423	ns
Coefficient of variation of double-limb support				
All subjects (n=54)	6.2±1.6	6.0±1.9	0.5029	ns
Responder (n=17)	5.9±1.8	6.0±2.4	0.7116	ns
Non-Resp. (n=30)	6.3±1.5	6.0±1.7	0.3138	ns
Coefficient of variation of base of support				
All subjects (n=54)	30±18	29±23	0.8942	ns
Responder (n=17)	30±20	32±37	0.6668	ns
Non-Resp. (n=30)	30±18	29±15	0.7748	ns
Coefficient of variation of length of C7 trajectory				
All subjects (n=54)	9.5±3.6	9.0±2.8	0.2810	ns
Responder (n=17)	10.2±4.5	9.0±2.8	0.2042	ns
Non-Resp. (n=30)	9.2±2.9	9.2±3.0	0.9473	ns

Measures of stability and trunk movements				
	Placebo	**Fampridine**	***P*-value**	**Sig.**
Double-limb support (% of step cycle)				
All subjects (n=54)	35±9	36±10	0.0091	ns
Responder (n=17)	35±8	36±8	0.1514	ns
Non-Resp. (n=30)	34±9	34±9	0.0884	ns
Base of support (mm)				
All subjects (n=54)	117±40	115±44	0.5909	ns
Responder (n=17)	120±49	119±53	0.7696	ns
Non-Resp. (n=30)	118±35	117±38	0.7335	ns
Length of C7 trajectory (mm)				
All subjects (n=54)	226±83	220±91	0.1283	ns
Responder (n=17)	255±109	246±122	0.3123	ns
Non-Resp. (n=30)	206±57	194±54	0.0055	ns

Forces				
	Placebo	**Fampridine**	***P*-value**	**Sig.**
Force during stance MIL (AUC normalized to body weight)				
All subjects (n=49)	33±12	33±11	0.6840	ns
Responder (n=15)	37±18	36±17	0.3261	ns
Non-Resp. (n=27)	30±4	29±5	0.6277	ns
Force during stance LIL (AUC normalized to body weight)				
All subjects (n=49)	34±10	34±10	0.1266	ns
Responder (n=15)	39±14	37±13	0.0580	ns
Non-Resp. (n=27)	32±7	31±7	0.1941	ns

Joint excursions				
	Placebo	**Fampridine**	***P*-value**	**Sig.**
Hip range of motion MIL (degree)				
All subjects (n=54)	36±8	36±8	0.2799	ns
Responder (n=17)	38±7	38±7	0.9670	ns
Non-Resp. (n=30)	35±9	36±9	0.2257	ns
Hip range of motion LIL (degree)				
All subjects (n=54)	40±6	40±6	0.6125	ns
Responder (n=17)	41±7	42±7	0.3616	ns
Non-Resp. (n=30)	40±6	39±6	0.1793	ns
Knee range of motion MIL (degree)				
All subjects (n=54)	31±13	33±13	**<0.0001**	***sig***
Responder (n=17)	29±14	31±13	0.0458	ns
Non-Resp. (n=30)	33±14	35±13	**0.0005**	***sig***
Knee range of motion LIL (degree)				
All subjects (n=54)	39±9	41±9	**0.0006**	***sig***
Responder (n=17)	35±9	37±8	0.0578	ns
Non-Resp. (n=30)	42±8	44±8	0.0232	ns
Ankle range of motion MIL (degree)				
All subjects (n=54)	20±6	20±5	0.3792	ns
Responder (n=17)	21±7	21±7	0.9601	ns
Non-Resp. (n=30)	20±5	19±5	0.1424	ns
Ankle range of motion LIL (degree)				
All subjects (n=54)	22±7	21±7	0.3509	ns
Responder (n=17)	21±6	20±7	0.3199	ns
Non-Resp. (n=30)	22±7	22±7	0.5576	ns

Mean treadmill velocity in km/h (80% of T25FW)				
All subjects (n=54)	3.2±1.4			
Responder (n=17)	3.0±1.3			
Non-Resp. (n=30)	3.4±1.5		0.3136	ns

Supplementary Figures and Tables

Prolonged-release fampridine in multiple sclerosis: improved ambulation effected by changes in walking pattern
A Randomized Clinical Trial

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Content:

Supplementary Figures and Tables

Suppl. Table 1: Schedule of study assessments.

Visits	S	V0	V1	V2	V3	V4	V5	V 6	V7	V8	V9	V10	V11	V12
Days	-21 to -7	0	1 ± 1d	7 14 ± 1d	15 21 ± 2d	28 ± 2d	42 ± 3d	56 ± 1d	57 70 ± 1d	71 77 ± 2d	84 ± 2d	98 ± 3d	112 ± 1d	113 126 ± 3d
Weeks	-3 to -1	0	1	2	3	4	6	8	10	11	12	14	16	18
	at least -7d but no more than -21d prior to V0	start of first single-blind placebo dosing	last d of first single-blind placebo dosing	first d of first double-blind dosing			last d of first double-blind dosing	first d of second single-blind placebo dosing	last d of second single-blind placebo dosing	first d of second double-blind dosing			last d of second double-blind dosing	2-week post-dose visit, last visit
General assessments														
Informed consent	X													
Physical examination/EDSS	X													X
Exclusion/inclusion criteria	X													
Background, demography	X													
Medical history, MS history[1], Concomitant medication, history of falls	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs[2]	X	X	X		X			X		X			X	X
12-lead ECG	X													
Urine pregnancy test[3]	X			X					X					X
AE / SAE, MS relapse	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Laboratory safety assessments: hematology, blood chemistry, urine analysis, creatinine clearance	X		X	X	X			X	X	X		X	X	X
Functional tests														
T25FW[4]		X	X	X	X	X	X	X	X	X	X	X	X	X
6MWT[4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TUG [4]					X		X		X	X	X	X		
BBS					X		X			X		X		
DGI					X		X			X		X		
Gait analysis[5]				X[6]		X		X			X		X	X
Additional assessments														
LEMMT / BMRC of LE muscle groups						X		X			X		X	
Actimeter							on		off			on		off
12-item WS	X							X					X	X
Fatigue questionnaire (WEIMuS)	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Footnotes: 1. MS history includes type of MS, clinical course of MS, current MS signs and symptoms and MS treatment history. 2. Vital signs include supine systolic and diastolic blood pressure, pulse rate and body temperature. 3. Only in women with childbearing potential. 4. The T25FW was performed twice with assistance (if required) and twice without assistance (if possible). The TUG was performed once with assistance (if required) and once without assistance (if possible). The 6MWT was performed only once, either with or without assistance. Subjects using an assistive device during the first tests (i.e., at the screening visit) continued to use the same device for all subsequent tests. 5. The walking speed on the treadmill was based on the individual maximal walking speed measured in the T25FW at visit 0 and 1 (80%). 6. Test run to familiarize subjects with the setup without assessment of gait parameters.

Abbreviations: S, screening; V, visit; d, day; EDSS, expanded disability status scale; MS, multiple sclerosis; ECG, electrocardiogram; AE, adverse events; SAE, serious adverse events; T25FW, timed 25-foot walk test; 6MWT, 6-minute walk test; TUG, timed up-and-go test; BBS, Berg balance scale; DGI, dynamic gait index; BMRC, British Medical Research Council scale; LE, lower extremity; 12-item WS, 12-item multiple sclerosis walking scale; WEIMuS, Wurzburg Fatigue Inventory for Multiple Sclerosis.

Supplementary Figures and Tables

Suppl. Table 2. Variability between measurements for the assessed kinematic parameters.

Parameter		Mean ± SD Placebo (%)*	Mean ± SD Fampridine (%)*	Sig.**	Mean ± SD Total (%)
Spatio-temporal gait parameters	Stance phase MIL	1.2 ± 1.0	1.5 ± 1.6	ns	1.3 ± 1.3
	Stance phase LIL	1.2 ± 1.4	1.4 ± 1.9	ns	1.3 ± 1.7
	Step length MIL	4.6 ± 4.3	4.6 ± 4.3	ns	4.6 ± 4.3
	Step length LIL	4.0 ± 3.7	5.2 ± 5.1	ns	4.6 ± 4.5
	Length of toe trajectory MIL	3.9 ± 3.1	4.9 ± 4.4	ns	4.4 ± 3.8
	Length of toe trajectory LIL	3.2 ± 3.1	4.6 ± 4.2	ns	3.9 ± 3.7
	Toe height MIL	12.9 ± 10.1	17.2 ± 15.0	ns	15.1 ± 12.9
	Toe height LIL	11.6 ± 11.0	13.0 ± 11.7	ns	12.3 ± 11.3
Stability	Base of support	13.6 ± 9.7	12.2 ± 9.2	ns	12.9 ± 9.5
	Length of C7 trajectory	7.4 ± 7.2	8.4 ± 8.0	ns	7.9 ± 7.6
	Double-limb support	3.4 ± 2.7	3.2 ± 3.9	ns	3.3 ± 3.3
Joint excursions	Hip range of motion MIL	6.8 ± 5.1	9.5 ± 7.4	ns	8.1 ± 6.4
	Hip range of motion LIL	5.9 ± 5.4	7.6 ± 6.2	ns	6.8 ± 5.8
	Knee range of motion MIL	12.7 ± 12.5	11.0 ± 11.1	ns	11.9 ± 11.8
	Knee range of motion LIL	7.5 ± 8.3	7.0 ± 7.0	ns	7.2 ± 7.6
	Ankle range of motion MIL	9.8 ± 6.5	8.9 ± 7.1	ns	9.4 ± 6.8
	Ankle range of motion LIL	9.0 ± 7.2	10.3 ± 8.7	ns	9.7 ± 8.0
Measures of variability	COV Length of toe trajectory MIL	28.2 ± 14.7	23.0 ± 15.9	ns	25.6 ± 16.8
	COV Length of toe trajectory LIL	24.4 ± 17.1	25.4 ± 14.7	ns	24.9 ± 15.9
	COV Double-limb support	28.6 ± 15.4	24.9 ± 19.2	ns	26.8 ± 17.4
	COV Base of support	20.7 ± 15.2	20.2 ± 13.9	ns	20.5 ± 14.5
	COV Length of C7 trajectory	22.6 ± 17.7	22.8 ± 13.8	ns	22.7 ± 15.8

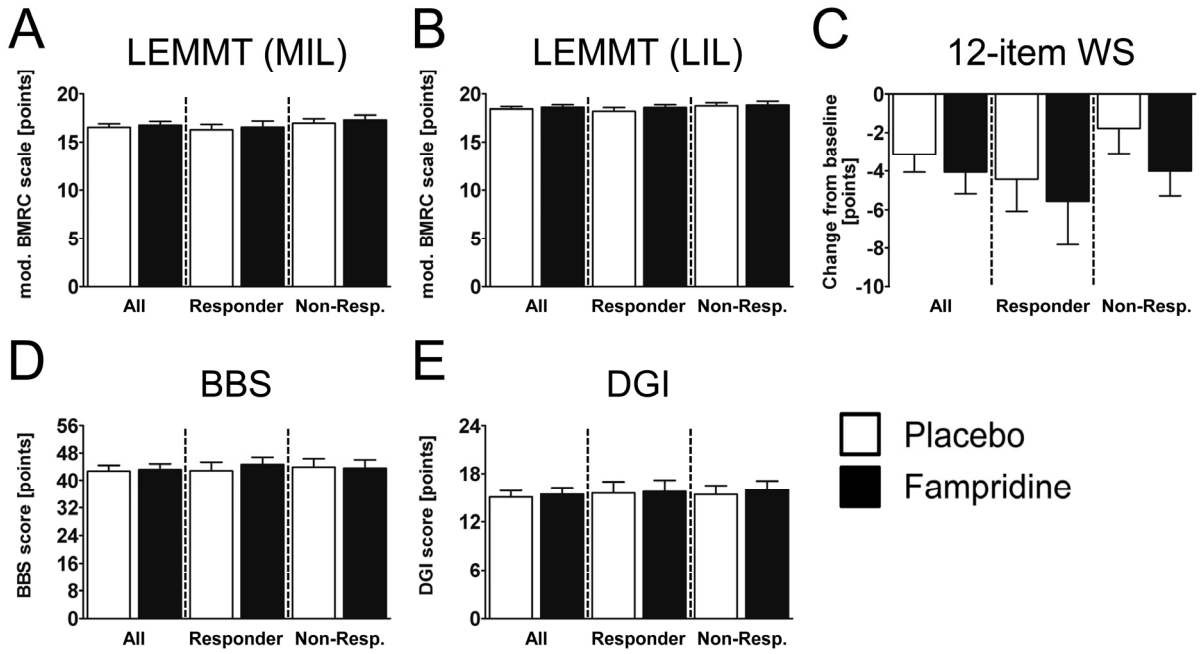
* Group average of changes (absolute value) in % between two measurements within one treatment period

** Result of paired *t*-test (placebo vs. Fampridine) after Bonferroni correction.

Difference (in percent) between the two measurements within the same treatment period for the placebo- and PR-fampridine phase separately (gray numbers). Note that there was no significant difference of the variability between measurements (measurement error) for the two treatment periods for all parameters. Therefore, total mean values (black) derived from averaging the results for both treatment periods were used to estimate the general variability between measurements for the kinematic parameters. Abbreviations: Sig., significant; ns, not significant; MIL, more impaired leg; LIL, less impaired leg, CoV, coefficient of variation.

Supplementary Figures and Tables

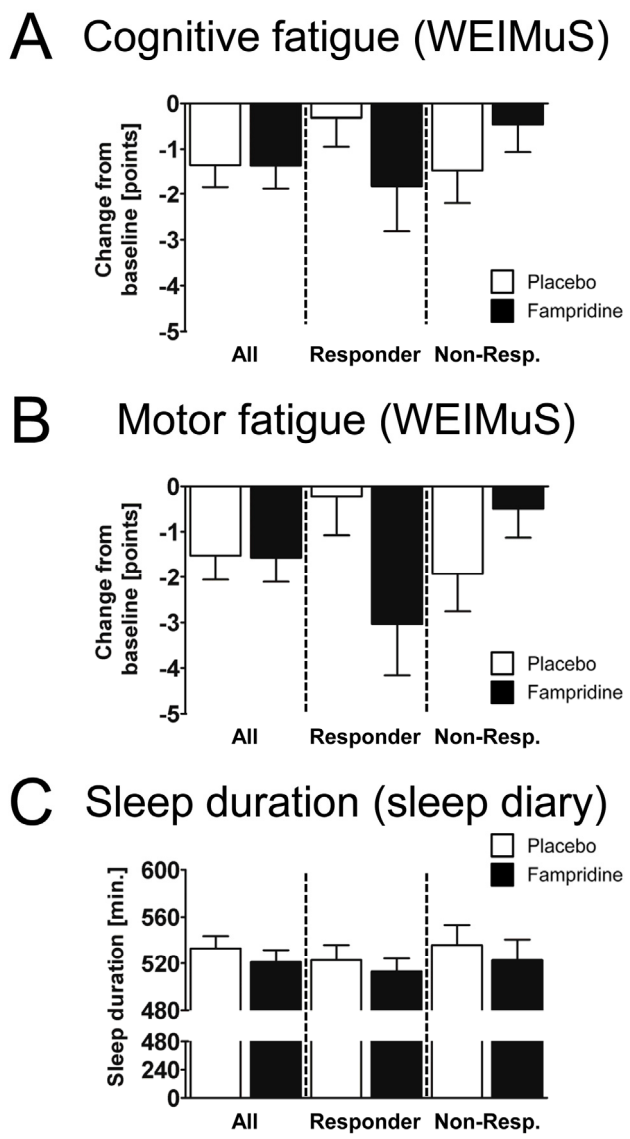
Suppl. Figure 1: Additional clinical tests and walking questionnaire.



(A-E) Results of LEMMT, 12-item WS, BBS, and DGI for all patients (n=55), responders (n=17) and non-responders (n=30). Note that for the 12-item WS, a score reduction indicates improvement. Legend illustrates color code for placebo (white) and PR-fampridine treatment (black). Bars show group mean values ± standard error of the mean. Abbreviations: Non-Resp., non-responder; LEMMT, lower extremity manual muscle test; MIL, more impaired leg; LIL, less impaired leg; 12-item WS, 12-item multiple sclerosis walking scale; BBS, Berg balance scale; DGI, dynamic gait index; mod. BMRC, modified British Medical Research Council.

Supplementary Figures and Tables

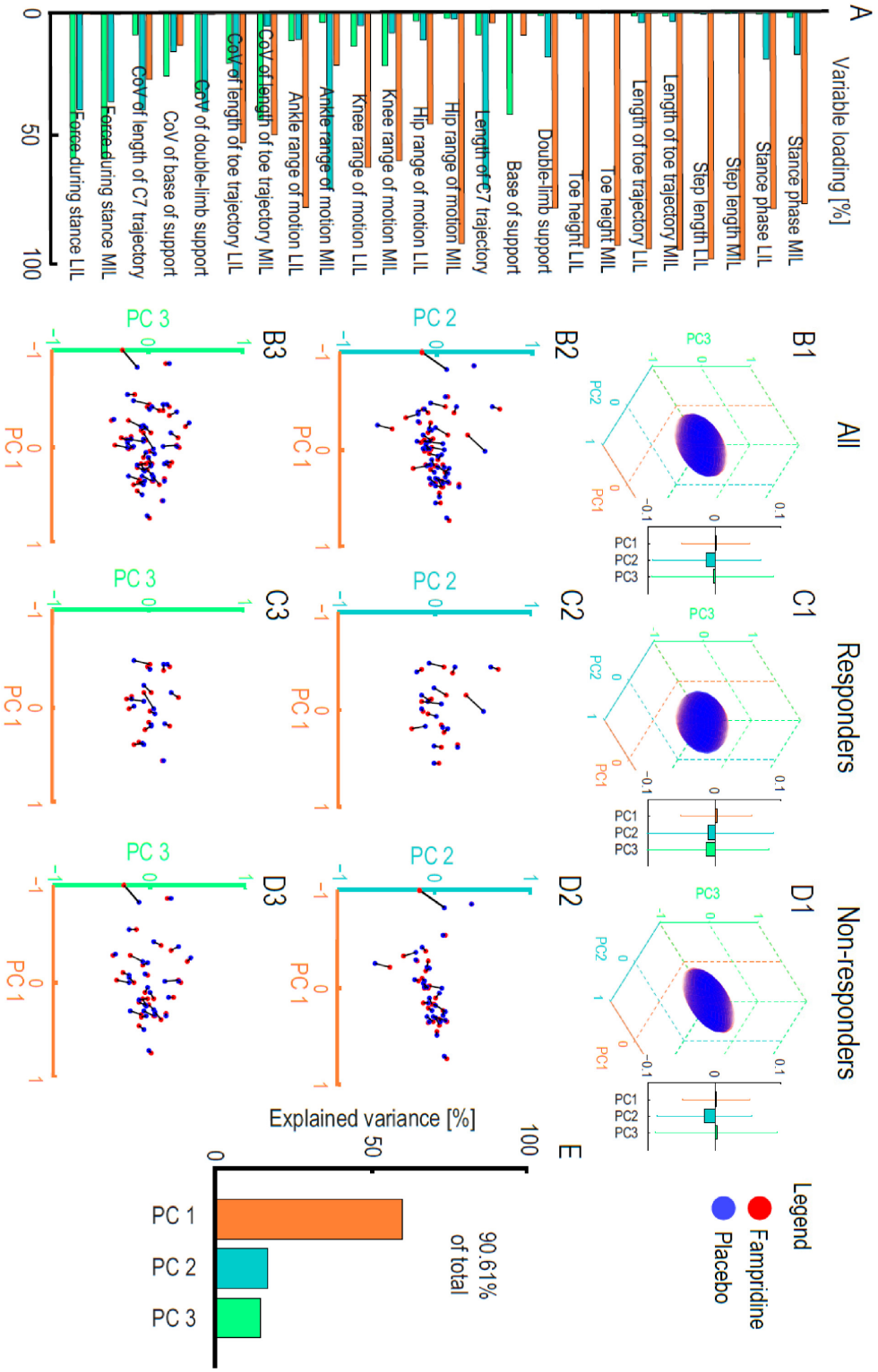
Suppl. Figure 2: Fatigue and sleep duration.



(A) Results for cognitive fatigue as assessed with the WEIMuS questionnaire. Changes from baseline are presented for all subjects (n=55), responders (n=17), and non-responders (n=30) for each of the two double-blind treatment periods. Note that a reduction (in points) indicates less fatigue. In all groups, there were no significant changes in cognitive fatigue during PR-fampridine treatment in comparison to placebo. (B) Results of motor fatigue as assessed with the WEIMuS questionnaire for patient groups as in (A). (C) Patients kept a sleep diary for 14 days during each of the two double-blind treatment periods. Mean sleep duration per day is presented in minutes for all subjects (n=54), responders (n=17), and non-responders (n=29). Bars show group mean values ± standard error of the mean. Abbreviations: Non-Resp., non-responder; WEIMuS, Wurzburg Fatigue Inventory for Multiple Sclerosis.

Supplementary Figures and Tables

Suppl. Figure 3: Principal components of kinematic and kinetic gait parameters.



(A) Specific loading of each variable on the three main principal components. Conditions are depicted with a grouping of all (B), responders (C) and non-responders (D) in the same PC-space. Series 1 shows a negligible effect of treatment (PR-fampridine vs. placebo) on location in PC-space independent of grouping. The associated bar graphs indicate non-significant mean translation in each dimension ± standard deviation. Series 2 and 3 illustrate this translation in two-dimensional PC-space for each individual and component. Note that the orientation of translation is unique for each data-pair. (E) Explained variance of the three applicable PCs identified by the stopping criteria. Abbreviations: PC, principal component; MIL, more impaired leg; LIL, less impaired leg; CoV, coefficient of variance.